



MAb Anti-phosphop44/42-Eu cryptate

PROTOCOL

Part # 61P10KAE, 61P10KAZ and 61P10KAY

Test size: 500 tests (61P10KAE), 10,000 tests (61P10KAZ), 100,000 tests (61P10KAY)

Assay volume: 20 μ L

Revision: 06 (Dec. 2017)

Store at $\leq -16^{\circ}\text{C}$

For research use only. Not for use in therapeutic or diagnostic procedures.

REAGENT DESCRIPTION

The Anti-Phospho p44/42 MAPK (Thr202/Tyr204) antibody is a monoclonal antibody. It recognizes p44 and p42 only when dually phosphorylated at Thr202 and Tyr204. The antibody does not cross-react with phosphorylated forms of SAP/JNK or p38 MAPK.

MATERIALS

Reagent	500 tests	10,000 tests	100,000 tests
MAb Anti-phosphop44/42-Eu cryptate. Frozen, in Hepes pH 7.0 containing protease free BSA	1 vial - 0.25 mL	1 vial - 0.25 mL	1 vial - 2.5 mL

Cisbio reagents - Not provided	Part #
HTRF KinEASE detection buffer 200 mL - ready-to-use	62SDBRDF
Plates - HTRF 96-well low volume plate	66PL96001

For HTRF microplate recommendations, please visit <http://www.cisbio.com/microplate-recommendations>

For reading, an HTRF[®]-certified reader is needed. Make sure to use the setup for Eu³⁺ Cryptate.

For a list of HTRF[®]- compatible readers and setup recommendations, please visit <http://www.cisbio.com/readers>

STORAGE AND STABILITY

- Store the reagents at $\leq -16^{\circ}\text{C}$.
- Under appropriate storage conditions, reagents are stable until the expiry date indicated on the batch information.
- Once thawed, stock solutions can be refrozen ($\leq -16^{\circ}\text{C}$) and thawed once only. Do not repeat freezing and thawing.

ASSAY FORMAT

When used as suggested, one vial from the three available sizes will provide sufficient reagent for 500 tests, 10,000 tests, and 100,000 tests respectively using a 20 μ L final assay volume.

Assay volumes can be adjusted proportionally to run the assay in 96- or 1536-well microplates.

	Volume
Other assay components	10 μ L
Acceptor (d2 or XL665) conjugate	5 μ L
Donor (Eu Cryptate) conjugate	5 μ L
Final volume	20 μ L

REAGENT HANDLING

BUFFERS

Cisbio KinEASE detection buffer (#62SDBRDF) has been optimized for maximum performance and is ready to use. When using specific in-house buffers for the preparation of working solutions, make sure to use a **phosphate-free buffer** (i.e. hepes), with a **pH maintained between 5.5 and 8.5**. It can be supplemented with BSA (0.1%), and detergents such as Tween 20, Triton X100 and CHAPS (up to 0.5%) to prevent reagent coating. Avoid SDS, due to its denaturing effect on XL665. Use of Europium antibody conjugate solution requires a final KF concentration between 100 mM and 400 mM. It is mandatory to use the same buffer to prepare the donor and the acceptor (d2 or XL665) conjugates.

CONJUGATES

Allow the stock solution to warm up at room temperature.

Prepare the conjugate solution according to the instructions included in the table below

MAB Anti-phosphop44/42-Eu cryptate*	Stock solution	Working solution preparation (see assay format)
500 tests	Ready to use Mix gently	Dilute 10-fold the stock solution in KinEASE detection buffer. Mix gently. E.g. Add 2.25 mL of KinEASE detection buffer to 0.25 mL of stock solution.
10,000 tests	Ready to use Mix gently	Dilute 200-fold the stock solution in KinEASE detection buffer. Mix gently. E.g. Add 49.75 mL of KinEASE detection buffer to 0.25 mL of stock solution.
100,000 tests	Ready to use Mix gently	Dilute 200-fold the stock solution in KinEASE detection buffer. Mix gently. E.g. Add 497.5 mL of KinEASE detection buffer to 2.5 mL of stock solution.

*Additional info is included on the batch information provided with the reagent.

Make sure to prepare stock and working solutions according to the instructions that correspond to the packaging you have purchased (number of tests).

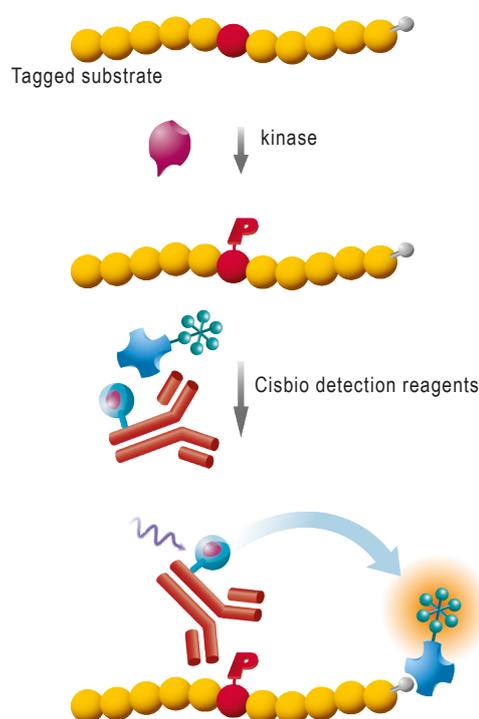
COMPANION REAGENTS

As illustrated beside, all kinase assays are based on the same format.

The enzymatic reaction is usually carried out with a biotinylated substrate (protein, peptide and the enzyme itself in the case of autophosphorylation).

The phosphorylated substrate is then detected using the specific anti phosphoresidue antibody coupled to Eu^{3+} Cryptate and a XL665 conjugate such as streptavidin-XL^{entl} (ref 611SAXLA) or Streptavidin-XL665 (ref 610SAXLA).

Alternatively, other tags such as GST-, 6HIS, c-myc or DNP may be used instead of biotin to label the kinase substrate.



This product contains material of biological origin. Use for research purposes only. Do not use in humans or for diagnostic purposes. The purchaser assumes all risk and responsibility concerning reception, handling and storage.

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